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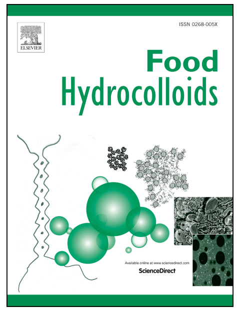

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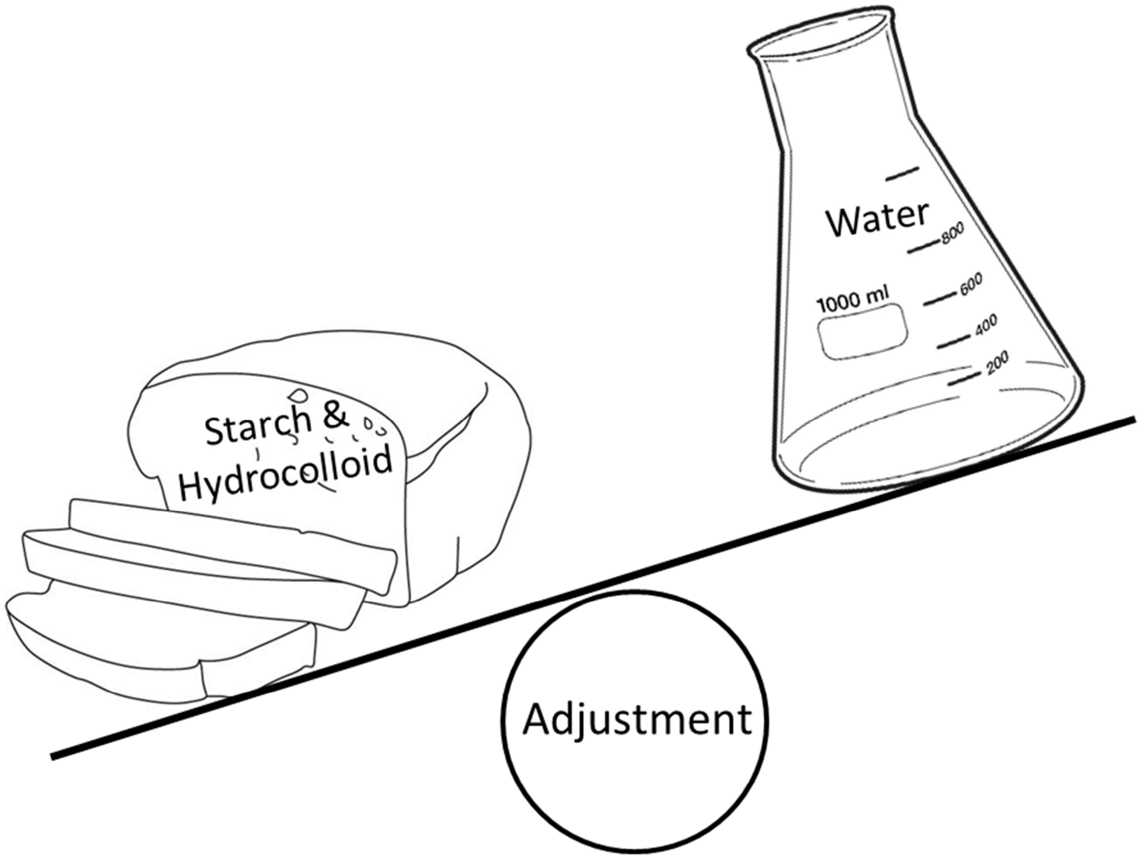

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Highlights:

- Molecular weight and charge of hydrocolloids had strongest effect on bread quality
- Negative charged hydrocolloids had low viscosity profiles due to repelling forces
- Sodium alginate reached highest specific loaf volume

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Water absorption as a prediction tool for the application of hydrocolloids in potato starch-based bread

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Abstract

To create visco-elastic networks in gluten-free doughs, hydrocolloids have been used most commonly to compensate for the lack of gluten. This study applies a prediction tool in form of an equation, considering the right water absorption level, to obtain optimised conditions for the use of six different hydrocolloids (guar gum, hydroxypropyl methyl cellulose, locust bean gum, pectin, sodium alginate, xanthan gum). For this purpose, the water holding capacity of each hydrocolloid was determined and the water amount in the formulation was adjusted accordingly to it. The hydrocolloids were analysed in five concentrations (0.25%, 0.5%, 1%, 1.5%, 2.0%). Analysis of water adjusted doughs included rheological properties, pasting properties and the baking performance. With the aid of the prediction tool, it was possible to obtain bread-like products for each hydrocolloid. However, the various hydrocolloids showed different concentration levels, where they performed best. In this study, the main influencing factors on bread quality were linked to the charge and the molecular weight of the various hydrocolloids. The negative charge of some hydrocolloids was hypothesised to created repelling forces between it and the negative charged phosphate groups of potato starches, affected those parameters. Bread baked with sodium alginate reached the highest specific volume at a concentration level of 1% and 2% xanthan gum had the softest bread crumb. Based on the source of used hydrocolloid, the analysis of the rheological and pasting properties revealed connections between dough properties and bread quality parameters.

1 Introduction

The production of high quality leavened baked gluten-free goods remains a technological challenge. The absence of gluten with its unique viscoelastic properties results in reduced gas retention and structure formation (Hager and Arendt 2013). A lot of research has been conducted to tackle this problem by the addition of hydrocolloids. They are water soluble polysaccharides with varied chemical structures and have a wide range of functional properties that make them suitable for different applications particularly in the area of gluten-free bread products (Li & Nie, 2016). Previously published literature related to gluten-free bread formulations state xanthan gum and hydroxy-propyl-methyl cellulose (HPMC) as the most used additives, amongst the hydrocolloids (Cato, Gan, Rafael, & Small, 2004; Hager & Arendt, 2013; Lee & Lee, 2006; Mancebo, San Miguel, Martínez, & Gómez, 2015; Sciarini, Ribotta, León, & Pérez, 2010; Sivaramakrishnan, Senge, & Chattopadhyay, 2004). The gluten-free market reflects this research showing that 40-70% of gluten-free breads contain xanthan gum and / or HPMC in their formulation, respectively (Foschia, Horstmann, Arendt, & Zannini, 2016). Hydrocolloids have now become a vital ingredient in the formulation of gluten-free breads. However, consumer demands are focused more and more on ingredient declaration. It is known that ingredients names like “xanthan gum” or “hydroxy-propyl-methyl cellulose” and their production background does not appeal to consumers. Hydrocolloids like guar gum, locust bean gum, pectin and sodium alginate could have the potential to replace xanthan gum and HPMC by keeping the quality of the product or even improve it. Locust bean gum and guar gum belong both to the family of galactomannans and are found in the carob and guar bean, respectively. Both galactomannans have a linear structure and a neutral charge. In comparison to other hydrocolloids, they have a wide range in size up to high molecular weights categorized from 50 kDa to 8,000 kDa and 50 kDa to 3,000 kDa, respectively (FAO 2017). Literature on the effect of locust bean gum in gluten-free bread formulations is scarce (Masure, Fierens, &

Delcour, 2016). Nevertheless, it was reported that a blend of locust bean gum and xanthan gum was more effective in improving dough structure and bread quality parameters, than locust bean gum on its own (Demirkesen, Mert, Sumnu, & Sahin, 2010). Also a recent study on the effect of xanthan gum and guar gum on gluten-free pan bread reported increased quality parameters when the hydrocolloids were blended (Gadallah, M. G. E., 2016). On the other hand, the application of guar gum on its own has recently been reported to improve quality and storage stability of gluten-free frozen dough (Asghar & Zia, 2016). Differences on the effect of hydrocolloids are assumed to be greatly influenced by the differences in formulation and occurring interactions. Pectin is mainly extracted from citrus peel. It consists of a linear chain with a molecular weight between 110 kDa and 150 kDa. It has been demonstrated to contribute to volume and structure in a gluten-free bread formulation (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007). Sodium alginate, a linear hydrocolloid (10 kDa to 600 kDa) with a negative charge is a structural component in marine brown algae. So far, it has only been incorporated in wheat-bread formulations where it was reported to have negative effects on volume and crumb hardness (Guarda, Rosell, Benedito, & Galotto, 2004; Rosell, Rojas, & Benedito de Barber, 2001). Guarda et al., (2004) stated that the properties of sodium alginate are very much depended on the extraction method and the source of algae.

This study provides a prediction tool in form of an equation. It considers the water holding capacity (WHC), to obtain optimised conditions for the use of six different hydrocolloids (guar gum, HPMC, locust bean gum, pectin, sodium alginate, xanthan gum) in gluten-free dough formulations. Table 1 gives a general overview about the important characteristics of them like their sources, molecular weights and charges. The objectives of this study were to compare these hydrocolloids and to test the tool in gluten-free bread formulations based on potato starch. For this purpose, the WHC of each hydrocolloid and potato starch was determined and the water amount in the dough formulation was adjusted accordingly. The hydrocolloids were analysed in 5 concentrations (0.25%, 0.5%, 1%, 1.5%, 2.0%). The obtained knowledge from this work is thought to contribute to the gluten-free product production and help to improve the knowledge and quality of gluten-free products.

2 Material and Methods

2.1 Material

Six commercially available hydrocolloids were used in this study. Guar gum and locust bean gum were obtained from Cargill, France; pectin and xanthan gum from Kelco, Germany; sodium alginate from Chemcolloids Ltd, Congleton, UK and HPMC by J. Rettenmaier & Söhne GmbH + Co. KG, Germany. Potato starch was supplied by Emsland, Germany; dry yeast by Puratos, Belgium; sugar by Siucra Nordzucker, Ireland; salt by Glacia British Salt Limited, UK.

2.2 Microscopy

Sample preparation of the doughs with the various hydrocolloids included the preparation of the dough (excluding yeast) and a freeze-drying process for 48 hours. The dough samples at 2% level of hydrocolloids were then cut and mortared. Both types of samples were then mounted on an aluminium stub, with the use of double-sided carbon tape. Samples were coated with a layer of 25 nm of sputtered palladium-gold. Hereupon, samples were examined under high vacuum in a field emission scanning electron microscope (JSM-5510 Scanning Electron Microscope, JEOL, München, Germany) with a working distance of 8 mm. Secondary electron images were acquired at an accelerating voltage of 5 kV. SEM Control User Interface software, Version 5.21 (JEOL Technics Ltd., Japan) was used for processing the images.

2.3 Water hydration capacity and water adjustment:

The measurement of WHC of above mentioned hydrocolloids was determined according to AACC method 56-30.01 with some modifications: samples (1.000g ± 0.005g) were mixed with 30 ml of distilled water using an Ultra-Turrax equipped with a S10N-5G dispersing element

(Ika-Labortechnik, Janke and Kunkel GmbH, Staufen, Germany) for 15 s and then shaken for 30 min at 1000 rpm using a platform shaker (UNI MAX 1010, Heidolph, Schwabach, Germany). Subsequently, the mixture was centrifuged at 2000 g for 10 min. WHC was expressed as ml of water retained per gram of solid:

$$\text{WHC [ml water / g ingredient]} = (W2-W1)/W0 \qquad \text{Eq. [1]}$$

Where W2 is the weight of the tube plus the sediment, W1 is the weight of the tube plus the sample and W0 is the sample weight.

The generated values where used in an equation to calculate and adjust the water content accordingly to the used hydrocolloid and its concentration.

$$\text{Water content [\%]} = (((a/100*c1) + (b/100*c2))*d)/e \qquad \text{Eq. [2]}$$

Where:
a = WHC of potato starch (= 0.590 ml/g)
b = WHC of Hydrocolloid
c1 = percentage of starch used in formulation based on dry ingredients (98.00 – 99.75)
c2 = percentage of hydrocolloid used in formulation based on dry ingredients (2.00 – 0.25)
d = 80% (based on starch) - optimal amount of water added to the base formulation (control)
e = 0.786 ml/g - combined WHC of the base formulation (potato starch 98 %and HPMC 2%; control).

The control values d and e were generated and calculated from previous research conducted on the impact of different starches on gluten-free formulations, here named as base formulation or control which contained 98% potato starch and 2% HPMC as solid base (Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016). Using Equation 2, the calculated percentages of water, were then applied in the various dough formulations throughout the study (Table 2).

2.4 Bread production

Bread samples were prepared according to (Horstmann et al., 2016). The formulation for the breads was as followed: 0.25 - 2% hydrocolloid, 2% salt, 4% sugar, 2% yeast, based on starch weight. The water addition depended on the used hydrocolloid and its concentration. Amounts were calculated as described in 2.3. Dry ingredients where mixed and yeast was suspended in warm water (25 °C) and regenerated for a period of 10 min. Mixing was carried out with a k-beater (Kenwood, Havant, UK) at low disk speed (level 1 of 3) for 1 minute in a Kenwood Major Titanium kmm 020 Mixer (Kenwood, Havant, UK). After the first mixing, the dough was scraped down from the bowl walls. A second mixing step of 2 minutes at higher disk speed (level 2 of 3) was applied. The batter was weighed (300 g) into baking tins of 16,5 cm x 11 cm x 7 cm and placed in a proofer (KOMA, Netherlands) for 45 min at 30 °C and 85% relatively humidity (RH). The proofed samples were then baked for 55 min at 220 °C top and 220 °C bottom heat in a deck oven (MIWE, Germany), previously steamed with 0.7 L of water. The breads were cooled for 2 hours prior to analysis.

2.5 Rapid visco analysis:

The pasting behaviours of the bread formulation (dry mix, excluding yeast) were measured according to the Newport Scientific Method 6, Version 4, December 1997, using a Rapid Visco Analyzer (RVA Super 3 Rapid Visco Analyser Newport Scientific, Warriewood, Australia). Samples were heated at a rate of 0.2 °C/sec from 50 °C to 95 °C, maintained at 95 °C for 162 s, cooled at the rate of 0.2 °C/sec to 50 °C, and held for 120 s at 50 °C before the test ended.

2.6 Viscoelastic properties of the dough

Oscillation measurements of dough samples (excluding yeast) were carried out by using a Rheometer Physica MCR 301 (Anton Paar GmbH, Ostfildern, Germany). Parallel serrated plates to prevent slippery, were used. The temperature of the lower plate was set to 30°C and used in conjunction with a 50 mm diameter upper plate. Frequency sweeps were conducted using a target strain of 0.01% and a frequency range from 100 to 0.1 Hz at 30°C. Before each test, the sample rested for five minutes to allow equilibration. Data obtained were complex modulus G* and the damping factor tan δ (G''/G').

2.7 Bread analysis:

Bread analysis was performed according to a previous work (Horstmann, Foschia, & Arendt, 2017). The specific volume of the bread was determined by a Vol-scan apparatus (Stable Micro System, UK. An image analysis system (Calibre Control International Ltd., UK) was used to analyse the bread crumb structure. Crumb texture was analysed using a Texture Profile Analyser (TA-XT2i, Stable Micro Systems, Godalming, UK) with a 25 kg load cell. Bread samples were sliced in 20 mm slices and analysed with a test speed of 5 mm/s and a trigger force of 25 g, compressing the middle of the bread crumb to 10 mm. Baked breads were stored in polythene bags (polystyrol-ethylene veniyl alcohol-polyethylene).

2.8 Statistical analysis

Results are reported as averages with standard deviation. Statistical analyses were performed with Minitab18 Software. A one-way ANOVA was conducted on the water holding capacity. Two way ANOVA was performed on the data of the viscosity and baking results affected by two experimental factors, hydrocolloid type and concentration. Holm–Sidak test was used to describe means at a 5% significance level. Correlation analysis was conducted to investigate correlations between the viscosity measurements and baking results.

3 Results & Discussion

In wheat dough formulations, the water is generally adjusted using the farinograph- method (AACC 54-21.02). This method allows to determine the exact amount of water, which is necessary to hydrate the dough and reach a set value measured in Brabender-Units (BU). The most commonly used value is 500 BU (Faubion & Hoseney, 1990). However, this method found also use for the prediction of water absorption in gluten-free bread formulations (Gujral & Rosell, 2004a, 2004b; Lazaridou et al., 2007; Sivaramakrishnan et al., 2004). These studies used flours and proteins in their formulations providing protein network and hydration. In this study, the farinograph showed limitations when the water additions were applied to the analysis of starch based gluten-free formulations containing hydrocolloids. These limitations are believed to be caused by the lack of protein and their network forming properties. A study by Hager & Arendt (2013) adjusted the optimal water content with the aid of response surface methodology. However, prior to the use of this tool preliminary trial-and-error baking test had to be conducted. None of the above methods are ideal and very often are very time consuming. Therefore, a need exists to develop a simple method to predict the water level in gluten-free formulations.

3.1 Water hydration capacity and water adjustment

The WHC determines the amount of water (in grams) bound per gram of hydrocolloids in an aqueous dispersion. In general, the WHC of ingredients used in food formulations play an important role, since it influences functional and sensory properties. The WHC showed significant different results between the various hydrocolloids (Table 1). Xanthan gum and guar gum showed the highest WHC indicating cold swelling properties, while sodium alginate and pectin had almost no swelling power demonstrating a high solubility and hot swelling properties. These characteristics are linked to the source of origin, chain length, molecular weight and distribution as well as polar charge of the hydrocolloid (Table 1) (Anton & Artfield, 2008; Capriles & Arêas, 2014). It is generally known that the polar charge has a high impact on the water affinity. Negatively charged hydrocolloids are more prone to build intermolecular hydrogen bonds with water, while uncharged hydrocolloids have intramolecular hydrogen bonds, which reduce the interactions with water. As stated in the literature also the chain length and the molecular weight affect the WHC of hydrocolloids. A study by Funami et al., (2005) correlated the molecular weight with the radius of gyrations, which is a measure for the distribution of components of an object around an axis, which in this study refers to water around the hydrocolloid. The study showed that the higher the molecular weight the higher the radius of gyration indicating a higher water holding capacity for hydrocolloids with a higher molecular weight. This can explain the low WHC for pectin and sodium alginate based on their low molecular weight. Furthermore, it justifies that xanthan gum despite its negative charge leads to a high WHC. These findings are in agreement with the earlier stated influencing factors on WHC in the literature. Additionally, it has been reported that a high number of branches increase the interactions with water. However, in this study only linear hydrocolloids were chosen and hence the factor of branching is neglected.

3.2 Scanning electron microscopy analysis

The micro structure investigation of the bread dough formulation (excluding yeast) is depicted in Figure 1. The images show the network formation of the hydrocolloids at a concentration of 2%. HPMC (b), locust bean gum (c) and to a certain extend guar gum (a) show thick strands expanding over the starch granules, forming a network. On the contrary, the dough formulation including sodium alginate shows a thin film coating the starch granules. Pectin (d) and xanthan gum (f) show mixture of film coating and particle strands covering the surface of the starch granules. The arrangement and thickness of strands is believed to have an influence on the dough properties regarding pasting and viscosity. This is in agreement with observations of Chaisawang & Suphantharika (2006). The authors found that xanthan gum molecules in contrast to guar gum coated the starch granules. This difference is thought to inhibit the granule swelling and reduce peak viscosity (Song, Kim, & Chang, 2006). The effect of hydrocolloids on starch was comprehensively reviewed by Bemiller (2011) and showed that a combination of hydrocolloid and starch could suppress the starch granule swelling and lower the viscosity. One of the explanations was the limited availability / accessibility of free water for the granules to swell.

3.3 Statistical analysis

3.4 Two way ANOVA was conducted on the pasting properties and baking results using multiple comparison of the two experimental factors concentration (with levels "0.25%"; "0.5%", "1.0%", "1.5%" and "2.0%") and hydrocolloid type (with levels "Locust bean gum", "Guar gum", "Sodium alginate", "Pectin", "HPMC" and "Xanthan"). Depending on the parameter measured different contribution levels of the concentration or the type of hydrocolloid were found. The contribution and significance levels of the various parameters is discussed in each individual paragraph. Pasting properties of dough formulations

The characteristics of starch granule swelling, breakdown and retrogradation during processing and storage determine the textures and stabilities of high moisture starch-based foods. These properties are attempted to be modified and or controlled by the addition of hydrocolloids (Bemiller, 2011). Starch is the main constituent in gluten-free products. Hence, its functional properties like pasting play a key role in the production of those.

Pasting properties (peak viscosity, breakdown viscosity and the peak time) of the various bread formulations are summarized in Table 3. Significant differences between the various hydrocolloids were observed. The different formulations, exhibit a range of properties like degrees of associations with other molecules of the same hydrocolloid and other molecules like water (Bemiller, 2011). Shi and Bemilller (2002) found that the molecules of the applied gums (CMC, carrageenan, alginate, xanthan) interact with leached amylose molecules, producing a viscosity increase via synergetic effects and prevent retrogradation. This increase in viscosity can be caused by hydrogen bonds created between the hydrocolloid and the leached amylose (Morris et al., 2008). Also, significant differences between the concertation level were expected as a higher concentration would strengthen the above-mentioned interactions. The peak viscosity is the point where starch granules swell to their maximum before they burst.

Two-way ANOVA indicated that the type of hydrocolloid is the main affecting parameter (79.03%, $p < 0.05$). The significant highest peak viscosities were reached by formulation containing locust bean gum and guar gum. The significant lowest viscosity was found in formulations containing sodium alginate. Overall it showed, that higher concentration of locust bean gum and guar gum, led to an increase in viscosity, whereas sodium alginate and pectin revealed a decrease in the viscosity with increasing levels. A similar effect was also observed by Kaur et al., (2008), who suggested that the decrease in viscosity in potato starch pastes was due to reduced granule swelling caused by the addition of cassia gum. In this study, lower viscosities by increasing levels of sodium alginate and pectin could be attributed to their negative charge. This negative charge can create repelling forces with the negatively charged phosphate groups on potato starch. Antagonistic forces restrict the pasting and gelatinization of starch granules, hence lowering the viscosity and delaying the pasting (Shi & BeMiller, 2002). In contrast to the other hydrocolloids HPMC and Xanthan at different concentrations did not affect the potato starch formulations viscosity. Song et al., (2006), reported that xanthan gum reduced the peak viscosity in potato starch, but found an increased viscosity in wheat starch. In this study, potato starch was used in combination with various hydrocolloids. Hence, it is believed that different interactions in comparison to wheat starch will occur. The starches of different origin leach different types of amylose, which in turn cause stronger or weaker interactions with applied hydrocolloids (Shi & BeMiller, 2002). In addition, it can be assumed that the coating of the starch granules, observed in the SEM micrographs (Figure 1), restrict the swelling leading to a decreased or maintained viscosity. The breakdown viscosity (BV), considered as an indicator for product stability to withstand heat and shear, also showed significant differences with the type of hydrocolloid as the main contributing factor (80.44%, $p<0.05$). The significant highest BV was found in formulations containing locust bean gum, while formulations with sodium alginate had the lowest. The data also showed a trend, where higher values for BV of locust bean gum and guar gum were measured with increasing hydrocolloid concentration, while sodium alginate, pectin and HPMC recorded a decrease in BV. Repeatedly, different concentration of xanthan gum did not change BV. The final viscosity (FV) is where recrystallization of the starch occurs and hence can be considered as an indicator for staling of cereal products. The applied two-way ANOVA test on the pasting properties revealed that the final viscosity was mainly influenced by the type of hydrocolloid (52.41%, $p<0.05$). Even though the contribution is not as high in comparison to the other parameters in can be seen that formulations with locust bean gum and HPMC showed the highest FV. The peak time (PT), which is the time to reach the peak viscosity, was delayed by the application and increasing concentration of sodium alginate,pectin and HPMC. Locust bean gum, xanthan gum and guar gum did not affect gelatinisation time. The main contributing factor affecting the peak time was also found to be the type of hydrocolloid applied (75.6%, $p<0.05$). It is hypothesised that a higher peak time, hence a delayed peak viscosity leads to a longer development of the bread structure before the setting occurs. In general, formulations including locust bean gum and guar gum showed the significant highest viscosity values followed by HPMC and xanthan gum. The lowest viscosity was found for sodium alginate and pectin. The effect of hydrocolloids on starch pastes and pasting behaviour has been intensively studied and been summarized in a literature review by BeMiller (2011). The literature cites over 250 studies, which conducted work on this topic and indicates that there is no general rule, which applies, when combining hydrocolloids with starches. Each combination of hydrocolloid and starch has different interactions.

3.5 Rheological studies:

Dynamic oscillatory measurements have been described to be non-destructive tests that measure the elastic (G’) and viscous (G’’) moduli by applying sinusoidal oscillating shear stress or strain over time, temperature, strain and frequency (Dobraszczyk & Morgenstern, 2003). Viscoelastic behaviour is an important characteristic of dough in order to facilitate gas /air cell expansion. Hydrocolloids have been reported to improve dough development and gas retention through an increase in viscosity, which in turn allowed the production of improved gluten-free breads (Capriles & Arêas, 2014). Figure 2 A and B display the effect of the chosen hydrocolloids at various concentrations on the viscoelastic properties of the bread dough (excluding yeast) over angular frequency. For all the doughs, it was observed that the increasing concentration of the hydrocolloid resulted in decreasing viscosity values. The major influencing factor for this is the higher amount of water added (Table 2) to the formulation. However, since the viscosity decrease was not proportional for all the hydrocolloids (e.g. xanthan gum), further factors such as the replacement of starch by hydrocolloids can have an influence on the lowered viscosity with increasing amounts of the hydrocolloids. Additionally, it is assumed that since the rheological measurements, different to the RVA measurements, which were conducted at low temperatures, the starches did not gelatinise and hence did not increase the viscosity. This effect is also described by Bemiller (2011), when preparing starch/hydrocolloid composite pastes or gels. Furthermore, a decrease in viscosity with higher frequency was observed, indicating a shear thinning effect. This shear thinning effect was also reported by other authors, when hydrocolloids were added to a bread formulation (Demirkesen et al., 2010; Gadallah, M. G. E., 2016; Kim, Patel, & Bemiller, 2013; Sivaramakrishnan et al., 2004). The behaviour of shear thinning is caused by the alignment of micro structure with the flow direction (Song et al., (2006). Demirkensen et al. (2010) stated, that the viscosity decreases, due to increasing shear, which leads to a break down molecular interaction.

The analysis of the damping factor is an indication of the visco- elastic behaviour. The dough formulations demonstrated rather elastic behaviour than viscous behaviour (G'>G''). Nevertheless, an increase in viscous behaviour was detected with increasing concentration of the hydrocolloids (except xanthan gum, Figure 2B). Repeatedly, this is mainly caused by the adjusted water content of the formulation. However, as also mentioned above further factors have to be taken into consideration. The exception of xanthan gum could be related to its higher molecular weight which is at least twice as high in comparison to pectin and sodium alginate. They showed the significant highest viscous behaviour values over the frequency of 8.73 [1/s] ($p<0.05$). It is hypothesised that the starch granules are restrained from swelling and hence do not develop elastic but rather viscous networks. It was observed that the increasing concentration levels of guar gum and xanthan gum did not affect the viscosity curve significantly. Due to the higher molecular weight of guar gum, it is assumed, that the highest viscosity level was already reached with the lowest concentration, therefore no viscosity changes were observed when the hydrocolloid concentration was increased. Xanthan gum is believed to have no effect on the viscosity profile with increasing concentration, this can be explained by the capability to coat starch granules (Figure 1). Even the lowest concentration of xanthan gum seems to be sufficient enough to retard the starch granule swelling.

A higher molecular weight, the distribution and the spatial arrangement would be able to form more complex aggregates through hydrogen bonds and polymer entanglements and therefore affecting the viscosity of the dough (Sciarini et al., 2010).

3.6 Baking performance of hydrocolloid containing formulations

Cross sections of the baked breads the different hydrocolloids at various concentrations are depicted in Figure 3. The illustrated bread slices allow a quick and broad overview of the differences in volume and cell structure. Overall, it can be seen that all the formulations revealed bread like products. This indicates that the calculation for the water adjustment was successfully applied as a prediction tool for hydrocolloids in this dough formulation. A more detailed description of the quality parameters is provided in Table 4.

Despite the water adjustment, the bread quality parameters show significant differences. This was already expected after the found significant differences in the pasting and rheological properties of the dough formulations. The two-way ANOVA revealed the type of hydrocolloid as the main contributor to the results of the specific volume (65.5%, $p<0.05$). It showed breads baked with sodium alginate reached the significant highest bread volume, while breads baked with locust bean gum reached the smallest volume. The one-way ANOVA in the individual hydrocolloid groups showed that an increasing concentration of hydrocolloid showed no significant effect on the specific volume for the formulations containing pectin, HPMC or xanthan gum. Whereas, locust bean gum, guar gum and sodium alginate showed significant differences in specific volume depending on the hydrocolloid concentration applied. It is worthwhile noting that an increased hydrocolloid concentration did not necessarily result in a higher bread volume. Guar gum and locust bean gum showed the opposite effect, reaching the highest loaf volume with the lowest concentration. Lazaridou et al.,(2007) showed that an increased concentration of xanthan gum, carboxyl methylcellulose, agarose and beta glucan in gluten-free bread formulations based on rice flour, corn starch and sodium caseinate reduced the loaf volume. It is hypothesised that the effect as described by Lazaridou et al.,(2007) is caused by the high molecular weights of the hydrocolloids applied. Based on the results presented in Table 4 the lowest concentration of guar gum and locust bean gum reached the ideal level of hydration and hydrogen bonding with the potato starch and the leached amylose. An increase in any higher concentration seems to create too strong interactions, possibly due to the discussed insufficient effect of the water addition (Section 3.4). Especially the decreasing effect of higher xanthan gum concentration on bread volume has been reported before (Crockett, Ie, & Vodovotz, 2011; Hager & Arendt, 2013; Sabanis & Tzia, 2011; Sciarini et al., 2010). Based on the significant differences in bread volume it was assumed that the bake loss would be also significant different, due to differences in the surface area. However, the bake loss of the various formulations did not show any significant differences across the entire range (data not shown). .

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Generated data only revealed relations between viscosity measured by the RVA and bread volume (r. -0.89, p<0.05). A higher viscosity of the dough supresses the gas cell expansion, hence leading to a smaller bread volume. The increasing concentration of hydrocolloids such as locust bean gum and guar gum increased the viscosity, while the increasing concentration of sodium alginate and pectin reduced it (Table 2). Additionally, it was found that doughs with a more viscous behaviour than elastic behaviour facilitated the gas cell expansion, leading to an increased specific volume. The differences in viscosity indicated some limitations of the applied method in relation to the analysis of the swelling properties of the various hydrocolloids and to use the generated data in the equation (Section 2.3). The applied method does not take the effect of the hydrocolloids when heated into consideration. Generated data on this effect could give more information about the performance of hydrocolloids during the baking process.

The factors; type of hydrocolloid (28.94%, p<0.05), concentration (45.46%, p<0.05) and interaction (19.89%, p<0.05) were indicated to contribute to the hardness values. However, the concentration was used as the main affecting factor. The post-comparison with the Holm-Sidak test resulting in groupings was performed on this basis. The grouping revealed that concentration levels of 2% resulted in the softest breads while the 0.25% resulted in the significant hardest breads. The authors assume that the higher amount of water added for higher concentrations of hydrocolloid and the replacement of the starch by more hydrocolloids lead to this trend. This would lower interactions between starch and hydrocolloids, reducing the retrogradation and recrystallization (Funami et al., 2005). The significant lowest hardness was found in bread containing xanthan gum and the highest hardness values was found in bread containing locust bean gum. The low hardness for xanthan gum breads is believed to be caused by the coating effect linked to its negative charge creating repelling forces and hindering the granules to swell and further retard the leaching of amylose. A reduced amount of leached amylose results in a reduced amount of retrograded amylose in the bread, which in turn leads to a softer crumb. Two-way ANOVA on the C-Cell parameters revealed low contribution levels for the type of hydrocolloid, the concentration and their interaction of the both, but high errors (data not shown). Hence it was not possible to draw clear conclusion on these parameters. The crumb structure parameters showed no significant differences for most of the hydrocolloids with increasing concentrations, except for locust bean gum. It showed a decrease in the number of cells with increasing concentration. This is assumed to be linked to the small loaf volume, leading to less cells than a higher bread volume.

In general it is known that different hydrocolloids affect gluten-free formulations to a different extents, based on their chemical structure, the amount used and interactions with other ingredients but also by process conditions (Hager & Arendt, 2013; Houben, Höchstötter, & Becker, 2012). By applying a two-way ANOVA test to our set of data, we found as well that most of the parameters were influenced by the type of which hydrocolloid was used. Only for the hardness of the bread crumb, the concentration of the various applied hydrocolloids was found to be the main contributing factor.

4 Conclusion

In this study the application of hydrocolloids (guar gum, HPMC, locust bean gum, pectin, sodium alginate, xanthan gum) at different concentrations (0.25%, 0.5%, 1.0%, 1.5%, 2.0%) in a gluten-free bread formulation based on potato starch was analysed. To facilitate this, a tool was developed to add the optimal water amount to the formulation, based on different water absorption properties of the hydrocolloids. All the hydrocolloid formulations resulted in bread like products. However, even though the different WHC of the hydrocolloids were considered and the water was accordingly adjusted, the breads showed significant differences and revealed different optimal hydrocolloid concentrations.

. In this study, the main influencing factor on bread quality was found to be the type of hydrocolloid used. This might be linked to the charge and the molecular weight of the various specific hydrocolloid. It is hypothesised, that sodium alginate and pectin due to their negative charge create repelling forces with the negative charged phosphate groups of potato starch. These antagonistic forces have a negative impact on the granule swelling, lower the viscosity and therefore allow gas cell expansion which results in higher bread volumes. In contrast to this, hydrocolloids like guar gum and locust bean gum do not create such repelling forces. Based on their high molecular weight and their neutral charge, it is hypothesised that many hydrogen bonds with leached amylose were created leading to high viscosity values. These high viscosity values lower the elasticity hence allowing only little gas cell expansion and ultimately lead to a smaller bread volume. This shows that the molecular weight had a stronger effect than the water. Hence, future research focusing on water absorption according to the molecular weight of the hydrocolloids is suggested. Also, the application of the prediction toll in a more complex system could give more insights of its applicability. The authors are confident to contribute to the knowledge in the gluten-free area, providing a new possibility to adjust the water content in a simple recipe containing hydrocolloids. In addition to this, the two-way ANOVA evaluation allowed to state that sodium alginate was the significantly best performing hydrocolloid in improving the bread quality parameters. It reached its maximum potential at a concentration level of 2%.

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Conflict of Interest:

The authors declare no conflict of interest.

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Table 1 Summarizing the important characteristics of the hydrocolloids used in this study including their measured water holding capacity.

Sample	Origin*	Structure*	Charge*	Chain length / Molecular mass*	Water holding capacity [g/ g sample weight]
Guar gum [E412]	Guar seed	Linear	Neutral	50 - 8,000 kDa	21.05 ± 0.63[a]
Hydroxypropyl-methyl cellulose [E464]	Modified cellulose	Linear	Neutral	13 – 200 kDa	10.39 ± 0.63[c]
Locust bean gum [E410]	Carob pot	Linear	Neutral	50 - 3,000 kDa	15.02 ± 1.46[b]
Pectin [E440]	Citrus peel	Linear	Negative	~100 kDa	4.65 ± 1.55[d]
Sodium Alginate [E401]	Brown algae	Linear	Negative	10- 600 kDa	4.63 ± 0.30[d]
Xanthan gum [E415]	Xanthomonas campestris	Linear	Negative	~ 1,000 kDa	18.72 ± 0.23[a]

- *Data sourced from fao.org [Accessed 15.8.2017] (FAO 2017)

Table 2 Percentages of water added to various formulation of different hydrocolloids at different concentrations

	Water addition based on solid (starch and hydrocolloid [%]				
Hydrocolloid Concentration	0.25%	0.50%	1.0%	1.5%	2.0%
Guar gum [E412]	65.25	70.46	80.87	91.28	101.69
Hydroxypropyl-methyl cellulose [E464]	62.54	65.04	70.02	75.01	80.00*
Locust bean gum [E410]	63.72	67.39	74.73	82.07	89.41
Pectin [E440]	61.30	62.55	65.50	67.57	70.15
Sodium Alginate [E401]	61.07	62.10	64.16	66.21	68.27
Xanthan gum [E415]	64.66	69.27	78.50	87.73	96.95

*Control recipe ((Horstmann et al., 2016)).

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Table 2 Pasting properties of various bread formulations

Properties	Peak 1 [RVU]	Breakdown [RVU]	Final Viscosity [RVU]	Peak Time [min]
Locust bean gum 2 %	2964.0 ± 2.0^{eA}	1097.7 ± 10.7^{eA}	2600.3 ± 22.1^{bA}	6.6 ± 0.1^{aD}
Locust bean gum 1.5 %	2716.7 ± 23.2^{dA}	912.3 ± 41.9^{dA}	2427.3 ± 33.5^{abA}	6.8 ± 0.1^{abD}
Locust bean gum 1.0 %	2477.0 ± 1.0^{cA}	777.3 ± 2.5^{cA}	2393.3 ± 35.4^{aA}	6.9 ± 0.0^{bcD}
Locust bean gum 0.5 %	2273.3 ± 30.6^{bA}	611.3 ± 68.3^{bA}	2328.0 ± 98.0^{aA}	7.0 ± 0.0^{bcD}
Locust bean gum 0.25 %	2141.7 ± 30.2^{aA}	492.3 ± 31.9^{aA}	2361.7 ± 100.9^{aA}	7.1 ± 0.1^{cD}
Guar gum 2%	2535.0 ± 136.8^{dB}	785.0 ± 44.0^{dB}	2424.7 ± 153.2^{aAB}	7.1 ± 0.0^{aD}
Guar gum 1.5 %	2473.0 ± 94.6^{cdB}	705.7 ± 46.1^{cdB}	2445.7 ± 86.5^{aAB}	7.1 ± 0.1^{aD}
Guar gum 1.0 %	2328.7 ± 20.2^{bcB}	661.0 ± 5.2^{cB}	2410.0 ± 22.3^{aAB}	7.0 ± 0.1^{aD}
Guar gum 0.5 %	2132.0 ± 30.8^{abB}	555.0 ± 42.7^{bB}	2245.0 ± 116.2^{aAB}	7.1 ± 0.1^{aD}
Guar gum 0.25 %	2059.7 ± 13.6^{aB}	459.0 ± 11.5^{aB}	2331.3 ± 16.9^{aAB}	7.1 ± 0.1^{aD}
Sodium alginate 2.0%	958 ± 2.6^{aE}	155.3 ± 5.5^{bF}	2035.7 ± 46.5^{aC}	8.9 ± 0.1^{cA}
Sodium alginate 1.5%	1049.3 ± 15.3^{abE}	142.7 ± 3.1^{abF}	2048.3 ± 5.5^{aC}	8.5 ± 0.2^{bcA}
Sodium alginate 1.0%	1215.3 ± 29.1^{bE}	124. 7 ± 3.1^{abF}	2110.7 ± 27.5^{aC}	8.9 ± 0.9^{bcA}
Sodium alginate 0.5%	1565.3 ± 102.8^{cE}	115.3 ± 4.6^{aF}	2229.3 ± 21.0^{bC}	7.7 ± 0.2^{abA}
Sodium alginate 0.25%	1717.3 ± 41.2^{cE}	210.0 ± 27.2^{cF}	2314.0 ± 35.4^{bC}	7.4 ± 0.0^{aA}
Pectin 2 %	1524.3 ± 16.1^{aD}	203.3 ± 7.7^{aE}	2060.0 ± 48.9^{aC}	7.6 ± 0.1^{bC}
Pectin 1.5 %	1520.0 ± 28.2^{aD}	190.3 ± 4.5^{aE}	2066.3 ± 17.2^{aC}	7.6 ± 0.1^{bC}
Pectin 1.0 %	1683.3 ± 7.2^{bD}	199.3 ± 4.0^{aE}	2191.0 ± 18.1^{bC}	7.5 ± 0.1^{abC}
Pectin 0.5 %	1867.7 ± 11.8^{cD}	311.7 ± 37.9^{bE}	2349.7 ± 22.7^{cC}	7.2 ± 0.1^{aC}
Pectin 0.25 %	1938.3 ± 37.1^{dD}	322.0 ± 21.1^{bE}	2351.3 ± 15.0^{cC}	7.2 ± 0.2^{aC}
HPMC 2%	1996.3 ± 8.6^{aC}	263.7 ± 45.5^{aD}	2419.0 ± 42.6^{aA}	7.9 ± 0.2^{bB}
HPMC 1.5 %	2024.0 ± 11.8^{abC}	283.3 ± 46.0^{abD}	2427.0 ± 39.5^{aA}	7.8 ± 0.2^{abB}
HPMC 1.0 %	1990.3 ± 8.4^{aC}	312.7 ± 10.0^{acD}	2368.7 ± 26.4^{aA}	7.8 ± 0.1^{abB}
HPMC 0.5 %	2021.3 ± 5.1^{aC}	360.7 ± 6.8^{bcD}	2384.3 ± 3.5^{aA}	7.6 ± 0.0^{abB}
HPMC 0.25 %	2060.3 ± 26.3^{bC}	387.7 ± 35.4^{cD}	2421.3 ± 35.1^{aA}	7.5 ± 0.1^{bB}
Xanthan 2%	2044 ± 4^{aC}	420.3 ± 49.6 ^{aC}	2279.0 ± 5.3 ^{aB}	6.7 ± 0.2 ^{aE}
Xanthan 1.5 %	1990.7 ± 18.6 ^{aC}	455.3 ± 23.7 ^{aC}	2278.7 ± 12.2 ^{aB}	6.5 ± 0.3 ^{aE}
Xanthan 1.0 %	1996.7 ± 45.2 ^{aC}	455 ± 11.3 ^{aC}	2342.0 ± 54.7 ^{aB}	6.3 ± 0.1 ^{aE}
Xanthan 0.5 %	2010.3 ± 40.8 ^{aC}	408.3 ± 46.7 ^{aC}	2373.0 ± 77.2 ^{aB}	6.3 ± 0.1 ^{aE}
Xanthan 0.25 %	1992.3 ± 28.3 ^{aC}	442 ± 15.4 ^{aC}	2320.0 ± 59.5 ^{aB}	6.5 ± 0.1 ^{aE}

Means in the same column for each individual hydrocolloid with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$). Results with different numbers are significantly different and grouped by two-way ANOVA. $^{(A-F)}$ type of hydrocolloid as main contributing factor; $^{(G-K)}$ concentration of applied hydrocolloid as main contributing factor.

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Table 3 Baking results of various hydrocolloid formulations

Baking properties	Specific Volume [g/L]	Hardness (baking day) [N]	Number of cells [-]	Number of cells/slice area [%]
Locust bean gum 2 %	2.7 ± 0.1^{aE}	11.33 ± 1.13^{aK}	2556.5 ± 121.0^{a}	0.53 ± 0.03^{a}
Locust bean gum 1.5 %	2.9 ± 0.1^{bE}	16.40 ± 0.82^{bI}	2829.1 ± 117.6^{ab}	0.56 ± 0.02^{a}
Locust bean gum 1.0 %	3.0 ± 0.1^{bcE}	15.33 ± 0.82^{bH}	2954.6 ± 171.4^{b}	0.57 ± 0.02^{a}
Locust bean gum 0.5 %	3.1 ± 0.0^{cE}	10.33 ± 0.68^{aJ}	2912.7 ± 89.6^{b}	0.53 ± 0.02^{a}
Locust bean gum 0.25 %	3.1 ± 0.0^{cE}	12.42 ± 0.93^{aG}	2988.1 ± 57.0^{b}	0.55 ± 0.01^{a}
Guar gum 2%	2.8 ± 0.0^{aDE}	5.40 ± 0.61^{aK}	2845.0 ± 92.8^{a}	0.58 ± 0.01^{b}
Guar gum 1.5 %	2.9 ± 0.0^{aDE}	9.74 ± 0.70^{bI}	2988.3 ± 95.7^{a}	0.59 ± 0.02^{b}
Guar gum 1.0 %	2.9 ± 0.0^{aDE}	13.32 ± 0.94^{dH}	2832.6 ± 158.3^{a}	0.55 ± 0.03^{ab}
Guar gum 0.5 %	3.2 ± 0.1^{bDE}	11.12 ± 0.69^{bcJ}	2962.1 ± 131.0^{a}	0.53 ± 0.03^{ab}
Guar gum 0.25 %	3.2 ± 0.1^{bDE}	12.70 ± 1.04 ^{cdG}	2916.7 ± 94.1^{a}	0.51 ± 0.02^{a}
Sodium alginate 2.0%	3.4 ± 0.1^{abA}	9.53 ± 0.61^{aK}	3021.7 ± 142.1^{a}	0.51 ± 0.02^{a}
Sodium alginate 1.5%	3.5 ± 0.1^{bA}	12.03 ± 0.67^{bcI}	3225.0 ± 248.6^{a}	0.52 ± 0.02^{a}
Sodium alginate 1.0%	3.6 ± 0.1^{bA}	12.95 ± 1.20^{bcH}	3078.5 ± 173.0^{a}	0.48 ± 0.02^{a}
Sodium alginate 0.5%	3.4 ± 0.0^{abA}	9.99 ± 0.76^{abJ}	2987.1 ± 253.9^{a}	0.48 ± 0.03^{a}
Sodium alginate 0.25%	3.3 ± 0.1^{aA}	14.50 ± 1.36^{cG}	3052.1 ± 178.38^{a}	0.52 ± 0.03^{a}
Pectin 2 %	3.4 ± 0.1^{aB}	7.22 ± 0.66^{aK}	3325.2 ± 543.47^{a}	0.54 ± 0.07^{a}
Pectin 1.5 %	3.3 ± 0.1^{aB}	9.92 ± 0.61^{abI}	2806.4 ± 107.51^{a}	0.48 ± 0.02^{a}
Pectin 1.0 %	3.4 ± 0.1^{aB}	11.76 ± 1.03^{bH}	2799.5 ± 109.82^{a}	0.48 ± 0.01^{a}
Pectin 0.5 %	3.4 ± 0.1^{aB}	10.76 ± 0.64^{bJ}	3080.7 ± 94.03^{a}	0.53 ± 0.02^{a}
Pectin 0.25 %	3.2 ± 0.1^{aB}	17.35 ± 1.96^{cG}	3036.3 ± 177.16^{a}	0.54 ± 0.02^{a}
HPMC 2%	3.1 ± 0.1^{aC}	8.39 ± 1.07^{aK}	2992.5 ± 190.76^{a}	0.55 ± 0.04^{a}
HPMC 1.5 %	3.3 ± 0.1^{aC}	11.57 ± 0.42^{bI}	2963.6 ± 102.70^{a}	0.53 ± 0.02^{a}
HPMC 1.0 %	3.2 ± 0.1^{aC}	14.94 ± 1.06^{cH}	2773.6 ± 112.16^{a}	0.50 ± 0.02^{a}
HPMC 0.5 %	3.2 ± 0.1^{aC}	10.31 ± 1.05^{abJ}	2760.3 ± 226.47^{a}	0.49 ± 0.03^{a}
HPMC 0.25 %	3.2 ± 0.1^{aC}	15.16 ± 1.67^{cG}	2758.4 ± 105.5^{a}	0.49 ± 0.03^{a}
Xanthan 2%	3.0 ± 0.1^{aD}	4.3 ± 0.43^{aK}	3039.4 ± 140.42^{a}	0.59 ± 0.03^{a}
Xanthan 1.5 %	3.0 ± 0.2^{aD}	6.58 ± 0.20^{bI}	3080.0 ± 128.87^{a}	0.58 ± 0.01^{a}
Xanthan 1.0 %	3.1 ± 0.1^{aD}	8.17 ± 0.57^{bH}	3052.7 ± 91.95^{a}	0.55 ± 0.01^{a}
Xanthan 0.5 %	3.1 ± 0.1^{aD}	7.97 ± 0.67^{bJ}	3081.2 ± 122.73^{a}	0.55 ± 0.02^{a}
Xanthan 0.25 %	3.1 ± 0.1^{aD}	11.43 ± 0.97^{cG}	3015.2 ± 141.53^{a}	0.55 ± 0.02^{a}

Means in the same column for each individual hydrocolloid with different letters are significantly different (≥3 = One-way ANOVA; ≥2 0 =t-Test, $p < 0.05$). Results with different numbers are significantly different and grouped by two-way ANOVA. $^{(A\text{-}F)}$ type of hydrocolloid as main contributing factor; $^{(G\text{-}K)}$ concentration of applied hydrocolloid as main contributing factor.

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Figure 1 SEM images of the various dough formulations (excluding yeast; 2% hydrocolloid). Magnification x2000. (a) guar gum; (b) HPMC; (c) locust bean gum; (d) pectin; (e) sodium alginate; (f) xanthan gum

Figure 2 Oscillation measurements on doughs prepared with the various hydrocolloids at different concentrations. A: Complex viscosity over frequency; B: tan delta (damping factor) over frequency

Figure 3 Cross sections of the baked breads with various hydrocolloids at different concentrations

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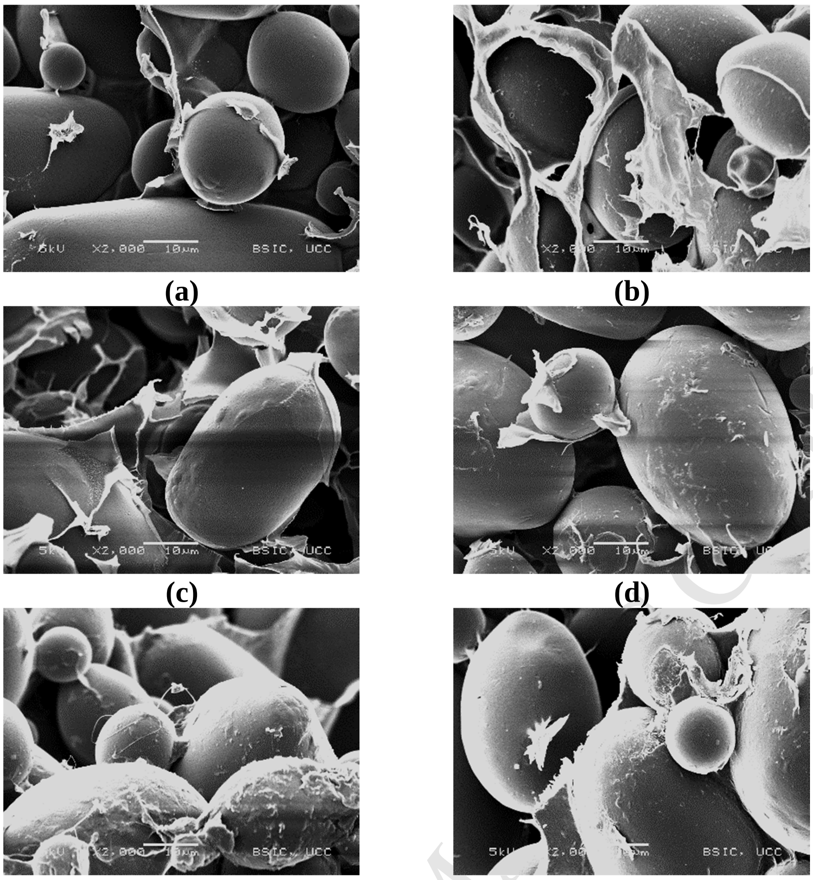
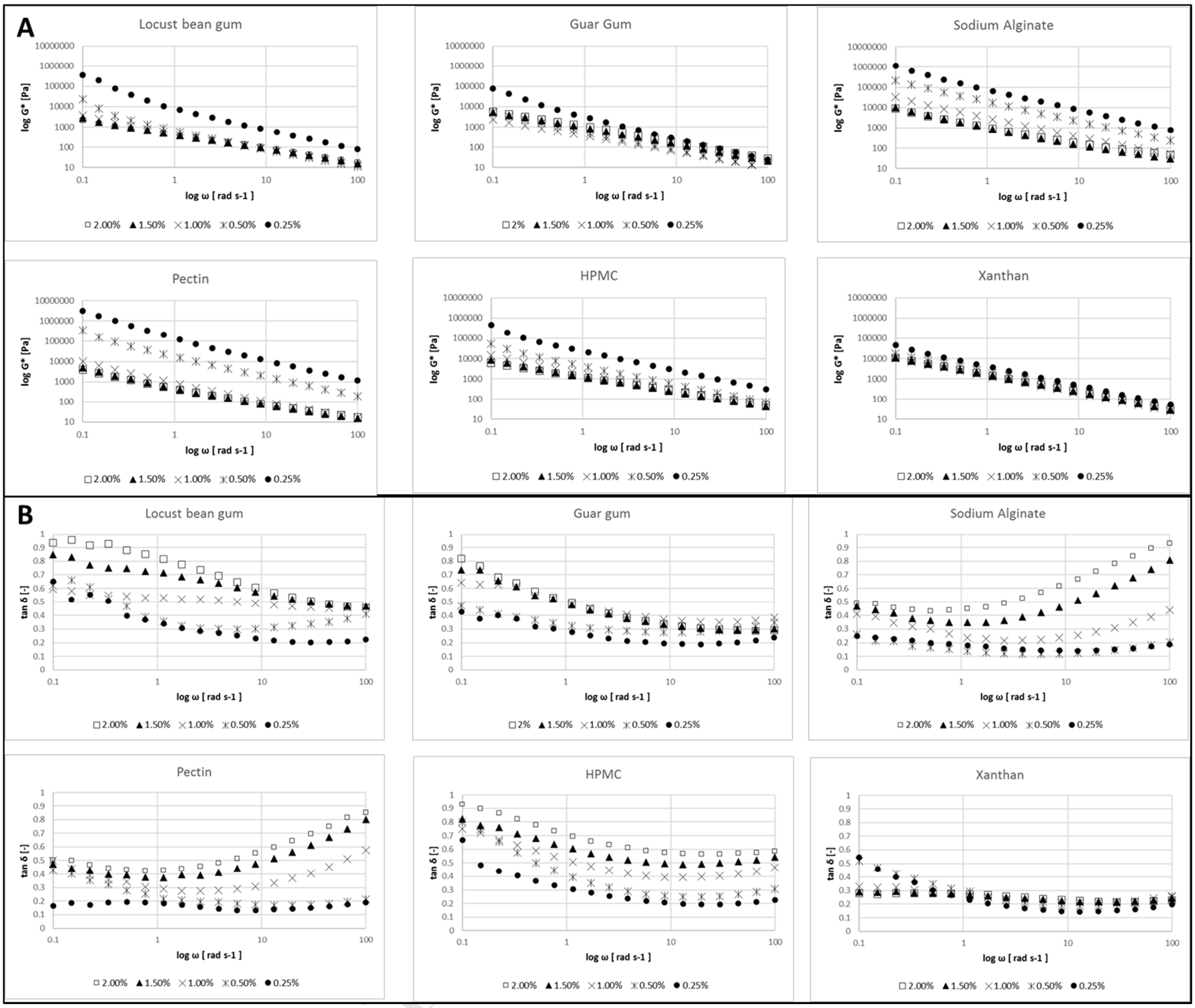


(a) (b)

(c) (d)

(e) (f)

ACCEPTED MANUSCRIPT



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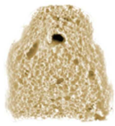
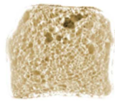
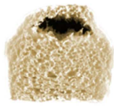
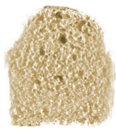
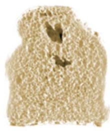
Sample / Concentration	0.25%	0.5%	1%	1.5%	2.0%
Guar gum					
HPMC					
Locust bean gum					
Pectin					
Sodium alginate					
Xanthan